NATURAL AND INDUCED IMMUNITY TO TYPHUS FEVER.*†‡

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Instances of transient or even permanent natural immunity of human beings to certain infections are not unknown. Most of these are clinical observations. It has been frequently noted that of a number of persons of a family or of a community presumably equally exposed, some escape. Those who have made many vaccinations for the prevention of smallpox, especially of children, are familiar with the fact that sometimes an individual may resist several repeated inoculations; this, while it may frequently be attributed to a non-potent virus, is in some instances, at least, undoubtedly due to a more or less transient natural immunity to vaccinia.

An interesting instance of natural immunity to an infectious disease in the human subject is reported by Reed.[1] One of the subjects experimented on by Reed and Carroll resisted a subcutaneous injection of 1.5 c.c. of yellow-fever blood and later resisted also the bites of some infected mosquitoes.

Similar examples of individual resistance of animals to experimental infection have been noted. In the course of our studies in measles we found quite a marked variation among monkeys in the susceptibility of different individuals to the disease. Marks[2] has well brought out the variation in susceptibility of young rabbits to poliomyelitis; and Dorset, McBryde, and Niles[3] report instances of natural immunity in the hog to hog cholera. Metchnikoff and

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‡ For protocols and fuller details see *Bulletin 86* of the Hygienic Laboratory, U.S. Public Health Service, on "Collected Studies on Typhus," paper No. 7, entitled "Studies in Immunity and Means of Transmission of Typhus," by John F. Anderson and Joseph Goldberger.

[1] *Med. Rec.*, 1901, 60, p. 201. [2] *Jour. Exper. Med.*, 1911, 14, p. 116.

[3] Bur. Animal Industry, U.S. Dept. of Agric., *Bull. 102*.

Besredka[1] report that in one of 16 experiments they failed to infect the chimpanzee with typhoid; and McCoy and Chapin[2] report that a considerable percentage of San Francisco rats were found by them to be immune to plague at a time when immunity, due to a previous attack, could reasonably be excluded. Coming to typhus, we find that Nicolle[3] was unsuccessful in his initial attempts to infect monkeys directly from the human subject, and from his experience concluded that to obtain success the virus had to be prefaced by passage through a higher ape, the chimpanzee.

Our own work[4] and that of Ricketts and Wilder[5], of Gaviño and Girard,[6] and others, and more recently that of Nicolle himself, has shown that such prefatory treatment of the virus is unnecessary. The apparently ready susceptibility of the monkey encountered by us in our early work suggested to us that Nicolle's initial lack of success was due to the small amount of blood used, to the subcutaneous route employed by him in those inoculations, or to both combined.

Apart from these factors there appeared to us no reason to suspect natural resistance as playing a part in explaining Nicolle's negative results.

Gaviño and Girard appear to have been the first to suggest that some monkeys may be naturally immune to typhus. They reported that a monkey of the species *Ateles vellerosus*, that had previously received an injection of heated typhus blood, failed to present any reaction following an immunity test of 5 c.c. of virulent blood for the control. They suggest that, instead of the animal having been vaccinated by the first non-infecting injection, it may have been naturally immune to typhus.

Dreyer[7] reports that in the course of his work he found one monkey (*Cercopithecus?*) entirely refractory. This animal showed

[1] Metchnikoff, E., and Besredka, A., *Ann. de l'Inst. Past.*, 1911, 22, p. 192; *ibid.*, 1911, 25, p. 865.

[2] U.S. Public Health and Marine Hospital Service, *Bull. 53.*

[3] Nicolle, Ch., and Conseil, E., *Compt. rend. de l'Acad. des sci.*, 1910, 151, p. 598; *Ann. de l'Inst. Past.*, 1911, 25, p. 13; Nicolle, Ch., Conor, and Conseil, *ibid.*, p. 97; Nicolle, Ch., and Conseil, *Compt. rend. de l'Acad. des sci.*, 1911, 153, p. 1522; Nicolle, Ch., Conseil, and Conor, *Ann. de l'Inst. Past.*, 1912, 26, p. 250; Nicolle, Ch., and Conseil, *ibid.*, p. 275; *ibid.*, pp. 332, 334.

[4] *Jour. Med. Res.*, 1910, 22, p. 469.

[5] *Arch. of Int. Med.*, 1910, 5, p. 361.

[6] *Publicaciones del Inst. bact. nacional Mexico*, November 9, 1910.

[7] *Arch. f. Schiffs- u. Tropen-Hyg.*, 1911, 15, p. 319.

no trace of illness, although two others treated with the same material at the same time and in substantially the same dose promptly developed the usual (typhus) symptoms. He believed himself qualified in interpreting this as a case of natural immunity. He does not state whether he tested the susceptibility of this animal a second time.

Wilder[1] reports that a monkey that had received an injection of filtered typhus blood serum failed to react when given an immunity test of 4 c.c. of typhus virulent blood and suggests, among other possibilities, that the animal may have been naturally immune.

Nicolle and his co-workers in their interpretations do not appear to have considered the possibility of a natural immunity in the monkey. We believe, however, that a critical study of their reported protocols shows that they had encountered the same phenomenon.

In the course of our recent work with the New York and the Mexican virus we obtained results that brought the possibility of the occurrence of a natural resistance to typhus forcibly to our attention.

We have summarized all our monkey inoculations in which the first or primary inoculation was with monkey typhus blood. The virulence of the blood used and the dose, as well as the route of inoculation employed, unless otherwise stated, was always proven. It was found that when 46 monkeys were given a primary inoculation of virulent defibrinated blood, eight or 17.4 per cent, failed to react. These eight were given a second inoculation, to which four or 8.7 per cent of the original 46 failed to respond. It is proper to state, however, that one of these four may perhaps be considered to have presented a slight suggestion of an "abortive" reaction—so slight, however, that it escaped our attention at the time of its occurrence. If we count this as a positive reaction, we have only three of the eight that failed to respond to a second inoculation; or, in other words, at least 6.5 per cent of the monkeys failed to respond to two separate inoculations with virulent blood.

Of the three clearly resistant to the second inoculation, the immunity of only two was further tested; of these, one—rhesus

[1] *Jour. Infect. Dis.*, 1911, 9, p. 101.

No. 197—although given less than half its previous dose now developed a mild though well defined febrile reaction; the other, rhesus No. 189, presented no indication of a reaction. After this rhesus No. 189 was subjected to five more tests. In all, between January 2 and June 29 this animal received eight inoculations with virulent blood, ranging in amount from 3 c.c. to 14 c.c., to which no recognizable evidence of a reaction was noted at any time during the period of observation. Of 46 animals, therefore, one (2.2 per cent) failed to respond to any of the eight inoculations to which it was subjected.

We have summarized our experiments in which the initial inoculation was either unfiltered or filtered serum.

Five monkeys received a first inoculation of virulent unfiltered serum; three of the five animals failed to respond to this inoculation. Of these three, two responded promptly to the first immunity test (second virulent inoculation), while one, rhesus No. 221, has so far resisted five successive immunity tests (six virulent inoculations).

Seven monkeys received a primary inoculation of filtered serum; none of the seven responded to this initial inoculation. When subjected to an immunity test, however, all but two (Nos. 115*a* and 194) responded; in other words, two of the seven failed to react to the first injection of virulent material. One of these two (No. 194) responded to the second immunity test and the other (No. 115*a*) to the third.

If now we combine the results summarized in the foregoing, we find that of 58 animals, 13 or 22.5 per cent failed to react (i.e., did not become infected) after one injection of virulent blood or blood serum; five or 8.5 per cent failed to react after two injections; and two or 3.5 per cent after three injections. It is evident that a very large proportion (22.5 per cent) of monkeys possess at least a transient immunity and it seems reasonable to consider that in about 3.5 per cent of animals the resistance noted amounts to a permanent immunity.

It may perhaps be objected that this is only an apparent immunity, that the resistance is simply due to a virus of low or varying virulence, to the smallness of the dose employed, the site of inoculation, or to the size or age of the animal.

It is readily conceivable that different strains of virus may differ markedly in virulence. Analogies readily suggest themselves, and the results of our inoculations with virus from human sources appear to furnish some experimental support for such a view. A summary of our inoculations with blood from human cases of typhus shows that only a small proportion of cases, whether New York or Mexican, were successful.

While it is not improbable that in these cases viruses of differing virulence may have entered as an element, we do not believe that this factor enters into the results of the series of inoculations above considered, for the inoculations in question were made with a single strain which we have successfully propagated through some 23 monkey generations, throughout which the virus, so far as we are able to discern, has maintained its original degree of virulence. The suggestion that age may enter as a factor in susceptibility has been advanced because it is held by some that children are less susceptible than adults. As a matter of fact, there is no good evidence to show that children are less susceptible to typhus than adults. It is probably true that the manifestations of typhus in a child are not in all respects like those in the adult; that is, they are not typical, but this manifestly cannot be regarded as indicating a difference in the degree of susceptibility. That this and the other objections cited are not valid may be clearly inferred from the fact, first, that of two monkeys of substantially the same size and vigor, both inoculated at the same time, by the same route but with different quantities of the same virus, the animal receiving the larger dose (by 50 per cent) has failed to react; and, second, as has already been noted above, an animal may react after the second or the third inoculation, although the dose may be only half as large as that given in the immediately preceding ineffective inoculation.

The existence of a more or less marked transient (or permanent) natural immunity is of great practical importance in all work on typhus. Great caution and conservatism must be observed in the interpretation of experimental results, especially negative results. We believe, too, that such immunity may have a much broader significance and application. It will be recalled that Metchnikoff and Besredka, in their studies on antityphoid vaccination conclude

that the ingestion of the bacillus paratyphoid-B may vaccinate against true typhoid. This conclusion is based on the observation that in one of two experiments, a chimpanzee that had previously been infected experimentally with paratyphoid-B was subsequently refractory to infection with typhoid. Now, while the results of future work may reinforce this conclusion the thought readily suggests itself that an occasional chimpanzee may be met with that may be transiently or permanently resistant to infection with typhoid. Indeed, these authors themselves, in a previous communication report that they met with only one failure in 16 experiments to infect anthropoids. We believe, therefore, that their conclusion is to some extent at least, if not altogether, invalidated.

In a discussion of the susceptibility of the monkey it is essential to have a clear understanding of what one is to consider as a tyhpus reaction in this animal. Following an inoculation with virulent material the monkey continues to remain normal for a period varying from five to 24 days. As may be seen from Table 1 in about 90 per cent of cases the incubation period varies between six and 10 days.

TABLE 1.

Incubation Period	Number of Monkeys
5 days	1
6	12
7	20
8	16
9	28
10	16
11	4
14	4
15	1
24	1
Total	103

At the end of this period the temperature of the susceptible animal rises fairly rapidly as a rule, sometimes gradually or at times very abruptly. The fever reaches its fastigium in 36 to 48 or 72 hours; it then continues for a variable period of one or two to five or more days, then defervesces. The defervescence, like the invasion, is variable. Although usually gradual, it is frequently rapid or even critical. In brief, the course of the fever in the monkey is essentially like that of the fever in man.

The fever may be accompanied by loss of appetite, thirst, a ruffling of the fur, and a drooping posture; very commonly, however, even with a well defined febrile reaction, the animal, except for some slight listlessness, shows hardly any outward manifestations. In other words, the fever is the only definite index of a reaction. As may be seen from Table 2, in about 76 per cent of the cases the fever varies in duration between six and 10 days.

TABLE 2.

Duration of Illness	Number of Monkeys
3 days	2
4	1
5	1
6	5
7	9
8	24
9	21
10	17
11	9
12	6
13	3
14	2
15	1
29	1
Total	102

At the termination of the fever there is almost always manifest some degree of emaciation. Occasionally after the temperature has been normal for three or four days or a week it may go up a second time. Such a relapse may last five or seven or more days, and may end in recovery or death. We have met with febrile recrudescense or relapse in four of 105 cases.

Although in appearance a mild disease, we have thus far had four deaths in a total of 105 cases of typhus in the monkey. This total of cases includes 10 induced with the Mexican virus. Segregating these, we have four deaths in 93 cases of the disease induced with the New York strain (Brill's disease), a mortality notably higher than that reported by Brill in the human subject.

An animal that has presented a reaction such as above described is immune from subsequent infection. In Table 3 we present the results of immunity tests in monkeys which had presented typical primary reactions. It will be seen that in no instance has such an animal responded to a subsequent immunity test. Such an immu-

nity may last a long time, as is shown by the following: "Adela," a female rhesus, was originally infected by an intraperitoneal injection of 6 c.c. of defibrinated blood from a patient with (Mexican) typhus, January 11, 1910. This animal developed a marked typhus terminating January 29–30, 1910. Between November 9, 1911, and March 6, 1912, this monkey was given four inoculations of typhus blood, all of which it resisted absolutely, showing that it was still immune after two years.

TABLE 3.

RESULTS OF IMMUNITY TESTS IN ANIMALS THAT HAD PREVIOUSLY PRESENTED A MARKED REACTION.

Rhesus Monkey No.	Date	Cubic Centimeters Inoculated	Source	Site	Result
95.....	Nov. 23, 1911	3	Rhesus 306	Vein	—
	Dec. 8	3.5	Case 19M*	Peritoneum	—
127.....	Dec. 30, 1911	6	Case 38M	"	—
	Jan. 1, 1912	4	Case 39M	"	—
133.....	Nov. 23, 1911	3	Rhesus 306	Vein	—
	Dec. 8	3.5	Case 19M	Peritoneum	—
142.....	Nov. 29, 1911	5	Rhesus 170	Vein	—
	Dec. 12	4	Rhesus 161	"	—
	Jan. 11, 1912	7	Rhesus 186	Peritoneum	—
158.....	Nov. 9, 1911	5	Case 1M	Vein and subcutaneous	—
	Dec. 2	2.5	Case 16M	Vein	—
	Dec. 22	4.5	Case 26M	Peritoneum	—
188.....	Mar. 30, 1912	3	Rhesus 234	"	—
210.....	"	3	"	"	—
306.....	Dec. 29, 1911	6	Case 35M	"	—
	Jan. 1, 1912	4	Case 39M	"	—
	Feb. 1	2.5	Rhesus 184	Vein	—
315.....	Feb. 1, 1912	2.5	"	Vein	—
316.....	"	2.5	"	Vein	—
	Feb. 23	6	Rhesus 200, 203, 204	Peritoneum	—
317.....	Jan. 10, 1912	3	Rhesus 187	Vein	—
318.....	"	3	"	Vein	—
500.....	Nov. 9, 1911	4	Case 1M	Vein	—
	Dec. 2	2.5	Case 16M	Vein	—
	Jan. 10, 1912	3	Rhesus 187	Vein	—
	Mar. 6	6	Rhesus 213, 312	Peritoneum	—

* M = Mexican case.

Ordinarily, therefore, a well marked febrile reaction may be interpreted as typhus without subjecting the animal to an immunity test. When, however, the fever is slight or its course atypical, that is, when we have what may be designated as an "abortive" fever, this cannot be construed as a typhus reaction unless the immunity test proves the animal to be resistant to infection and even then not without some reserve. Should the immunity test in such cases show that resistance has not been conferred, a diagnosis of previous typhus is not permissible.

CONCLUSIONS.

1. Instances of a transient or permanent natural immunity of the monkey to typhus are not uncommon. In our experience 22.5 per cent of monkeys have failed to react to a first and 3.5 per cent to three or more successive inoculations with virulent blood or blood serum.

2. Repeated inoculations of virulent blood or blood serum, when not followed by a febrile reaction, confer no appreciable resistance. Failure of an animal to react to an immunity test cannot, in the absence of a previous febrile reaction, be interpreted as indicating that it was protected (vaccinated) by the primary, apparently ineffective, inoculation.

3. A typical febrile reaction has, in our experience, always conferred complete protection to subsequent infection; in one instance for at least two years. Such a fever, therefore, under experimental conditions, justifies a diagnosis of typhus.

4. An atypical or poorly defined ("abortive") fever following inoculation cannot be interpreted as typhus unless it is followed by resistance to subsequent immunity tests.